Fig. 1

Mutant-specific oligonucleotide primers used for mutant number 1. Mutated nucleotide underlined

```
                    10        20        30        40        50
                    |         |         |         |         |
Bet v 1 sense       5'- AATTATGAGACTGAGACCACCTCTGTTATCCCAGCAGCTCG -3'
Bet v 1 non-sense   3'- TTAATACTCTGACTCTGGTGGAGACAATAGGGTCGTCGAGC -5'
sense primer        5'-           TGAGACCCCCTCTGTTATCCCAG         -3'
non-sense primer    3'-     ATACTCTGACTCTGGGGGAGACA               -5'
```

Fig. 2

Oligonucleotide primers for site directed mutagenesis of Bet v 1 (No. 2801).

all	sense	1: 183Bv, 15-mer	5'-GTTGCCAACGATCAG
1	sense	2: 184Bv, 23-mer	5'-TGAGACCCCCTCTGTTATCCCAG
1	non-sense	3: 185Bv, 23-mer	5'-ACAGAGGGGGTCTCAGTCTCATA
2	sense	4: 186Bv, 31-mer	5'-GATACCCTCTTTCCACAGGTTGCACCCCAAG
2	non-sense	5: 187Bv, 31-mer	5'-ACCTGTGGAAAGAGGGTATCGCCATCAAGGA
3	sense	6: 188Bv, 23-mer	5'-AACATTTCAGGAAATGGAGGGCC
3	non-sense	7: 189Bv, 23-mer	5'-TTTCCTGAAATGTTTTCAACACT
4	sense	8: 190Bv, 23-mer	5'-TTAAGAACATCAGCTTTCCCGAA
4	non-sense	9: 191Bv, 23-mer	5'-AGCTGATGTTCTTAATGGTTCCA
5	sense	10: 192Bv, 23-mer	5'-GGACCATGCAAACTTCAAATACA
5	non-sense	11: 193Bv, 23-mer	5'-AGTTTGCATGGTCCACCTCATCA
6	sense	12: 194Bv, 23-mer	5'-TTTCCCTCAGGCCTCCCTTTCAA
6	non-sense	13: 195Bv, 23-mer	5'-AGGCCTGAGGGAAAGCTGATGTT
7	sense	14: 196Bv, 24-mer	5'-TGAAGGATCTGGAGGGCCTGGAAC
7	non-sense	15: 197Bv, 24-mer	5'-CGCTCCAGATCCTTCAATGTTTTC
8	sense	16: 198Bv, 24-mer	5'-GGCAACTGGTGATGGAGGATCCAT
8	non-sense	17: 199Bv, 24-mer	5'-CCATCACCAGTTGCCACTATCTTT
all	non-sense	18: 200Bv, 15-mer	5'-CATGCCATCCGTAAG

Fig. 3

Overview of all mutations

```
                                  1(A-C)
GGTGTGTTTAATTATGAGACTGAGACCACCTCTGTTATCCCAGCAGCTCGACTGTTCAAG      60
 G  V  F  N  Y  E  T  E  T T-P S  V  I  P  A  A  R  L  F  K       20

            9(A-G)      2(A-C) 2(A-C)
GCCTTTATCCTTGATGGCGATAACCTCTTTCCAAAGGTTGCACCCCAAGCCATTAGCAGT      120
 A  F  I  L D-G G  D N-T L  F  P K-Q V  A  P  Q  A  I  S  S       40

      3(GA-TC)   7(AA-TC)                 4(G-C)      6(GA-TC)
GTTGAAAACATTGAAGGAAATGGAGGGCCTGGAACCATTAAGAAGATCAGCTTTCCCGAA      180
 V  E  N  I E-S G N-S G  G  P  G  T  I  K K-N I  S  F  P E-S      60

                                                 5(CA-TG)
GGCCTCCCTTTCAAGTACGTGAAGGACAGAGTTGATGAGGTGGACCACACAAACTTCAAA      240
 G  L  P  F  K  Y  V  K  D  R  V  D  E  V  D  H T-A N  F  K       80

TACAATTACAGCGTGATCGAGGGCGGTCCCATAGGCGACACATTGGAGAAGATCTCCAAC      300
 Y  N  Y  S  V  I  E  G  G  P  I  G  D  T  L  E  K  I  S  N      100

10(GAG-CAC)       8(CCC-TGG)
GAGATAAAGATAGTGGCAACCCCTGATGGAGGATCCATCTTGAAGATCAGCAACAAGTAC      360
 E  I  K  I  V  A  T P-G D  G  G  S  I  L  K  I  S  N  K  Y      120

CACACCAAAGGTGACCATGAGGTGAAGGCAGAGCAGGTTAAGGCAAGTAAAGAAATGGGC      420
 H  T  K  G  D  H  E  V  K  A  E  Q  V  K  A  S  K  E  M  G      140

GAGACACTTTTGAGGGCCGTTGAGAGCTACCTCTTGGCACACTCCGATGCCTACAACTAA      480
 E  T  L  L  R  A  V  E  S  Y  L  L  A  H  S  D  A  Y  N stop    159
```

FIG. 4

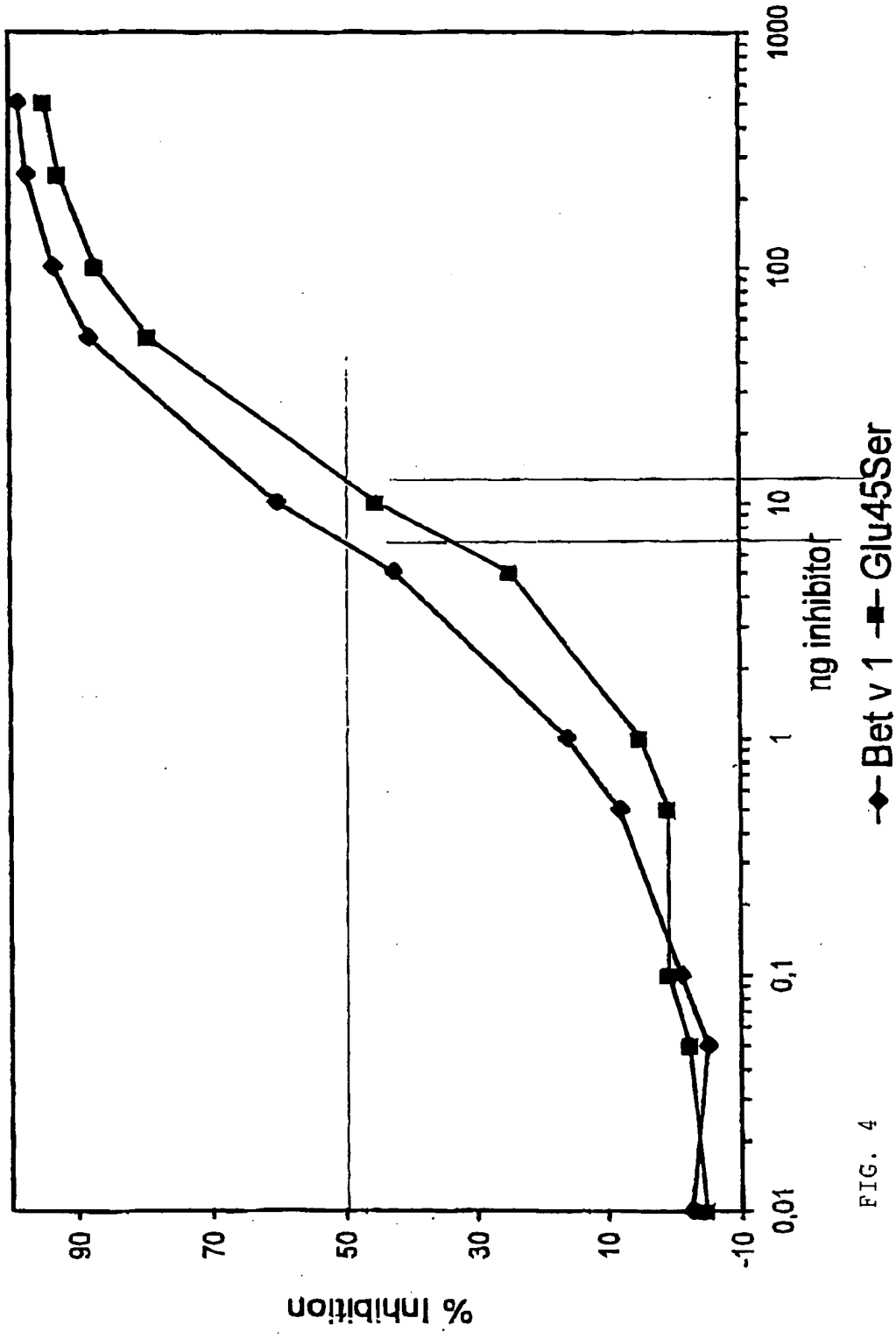

% Inhibition
90
70
50
30
10
-10
0,01
0,1
1
10
100
1000
ng inhibitor
Bet v 1
Glu45Ser

FIG. 4

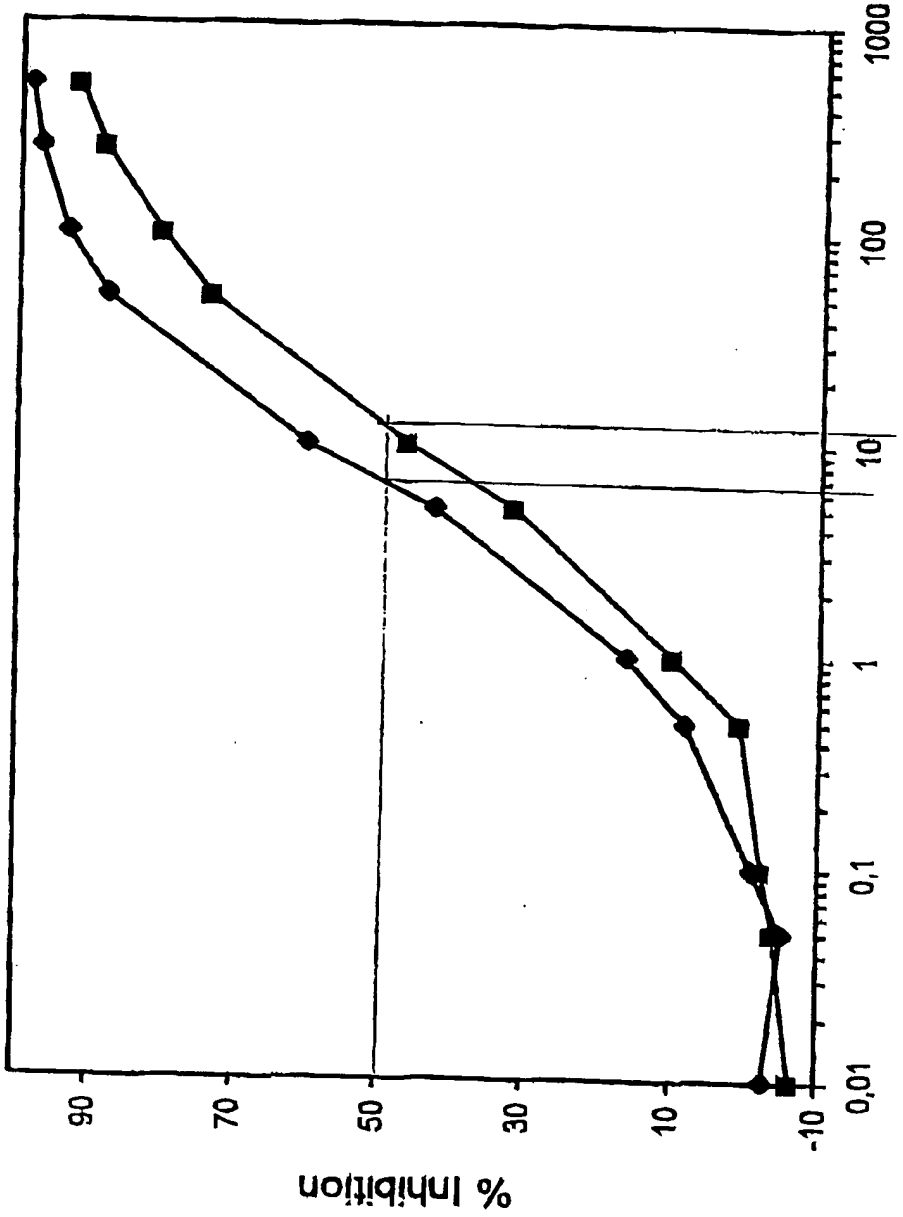


FIG. 5

Fig. 6

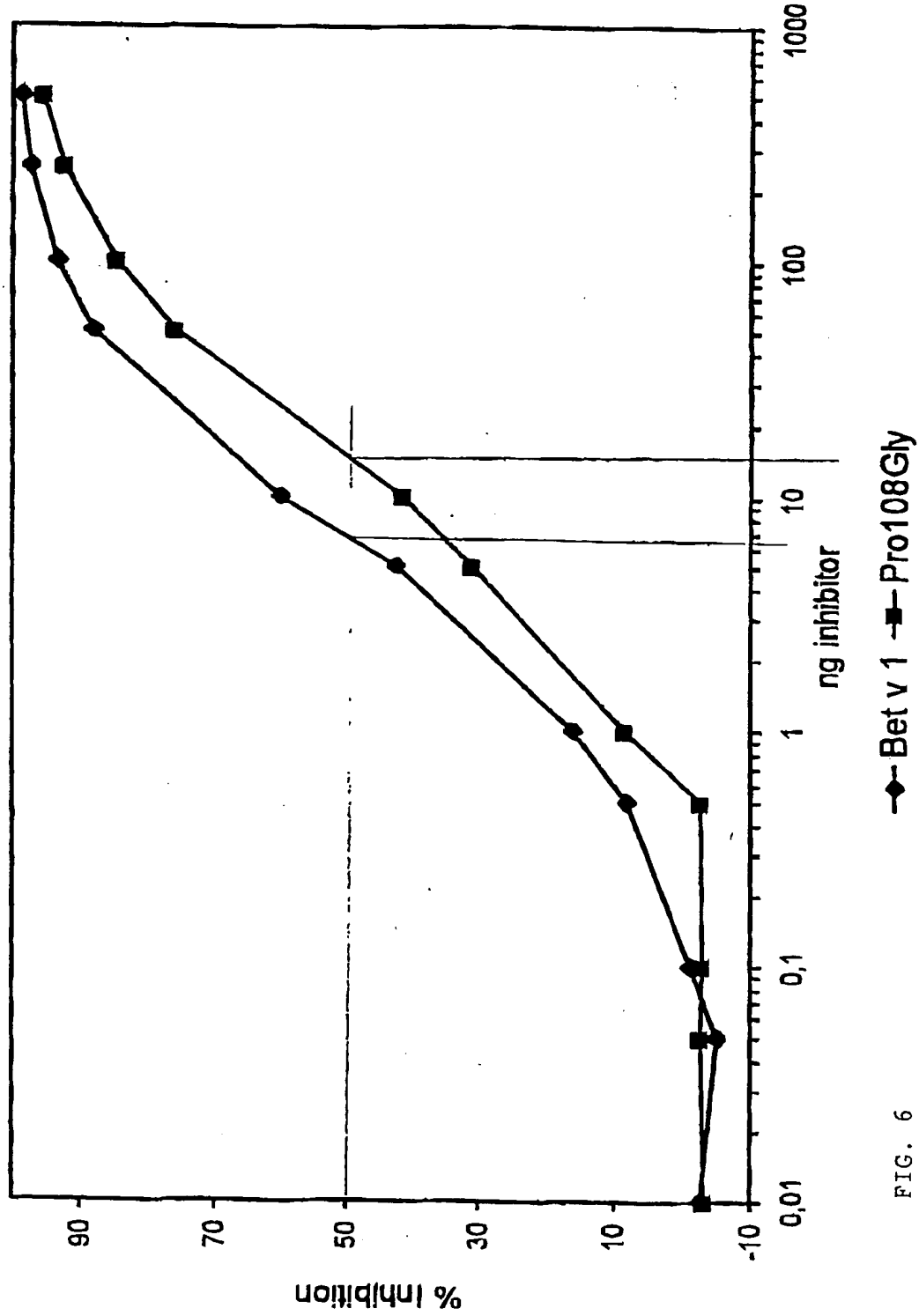

% inhibition
90
70
50
30
10
-10
0,01
0,1
1
10
100
1000
ng inhibitor
Bet v 1
Pro108Gly

FIG. 6

Fig 7

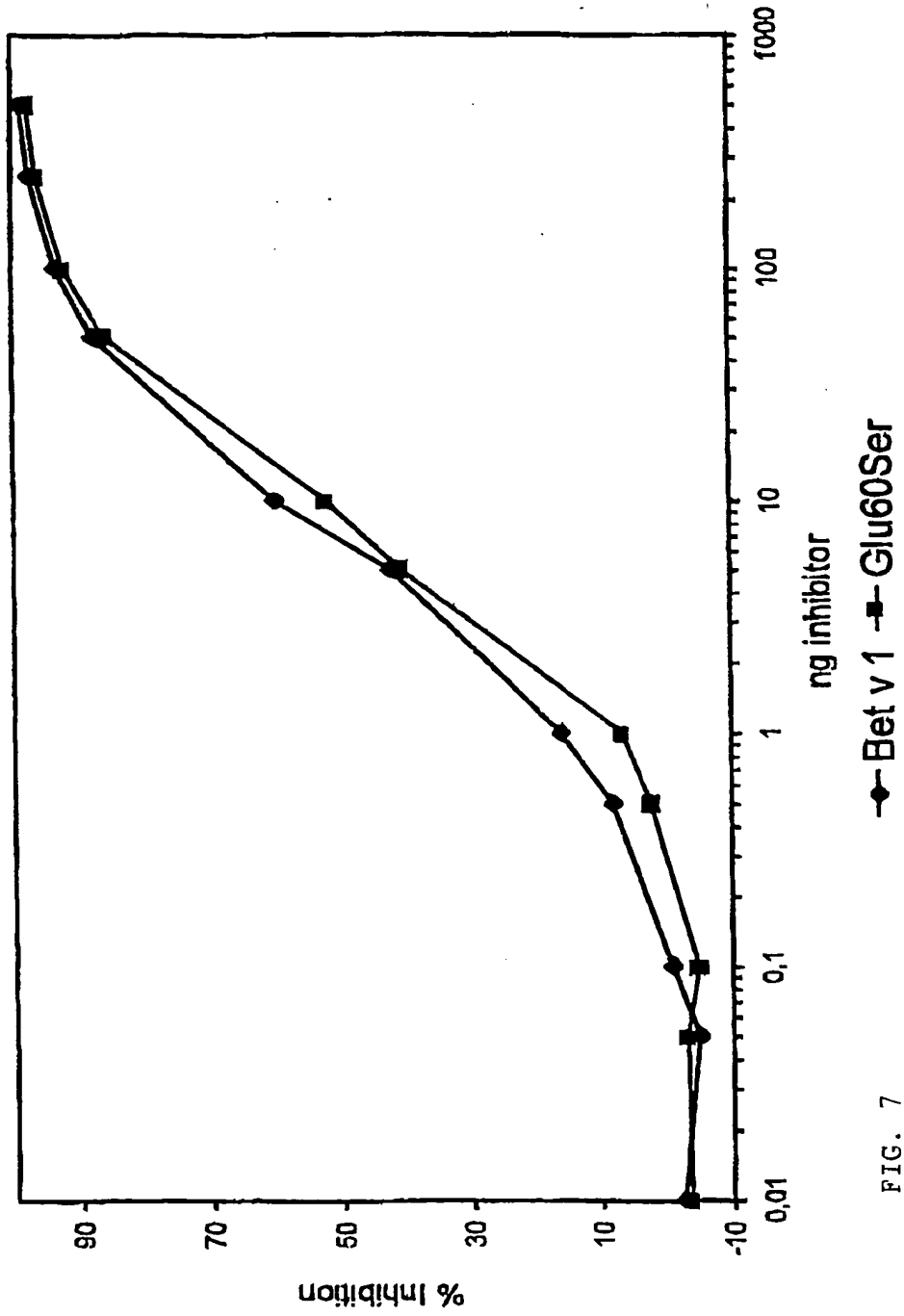

% Inhibition
90
70
50
30
10
-10
0,01
0,1
1
10
100
1000
ng inhibitor
Bet v 1
Glu60Ser

FIG. 7

Fig. 8

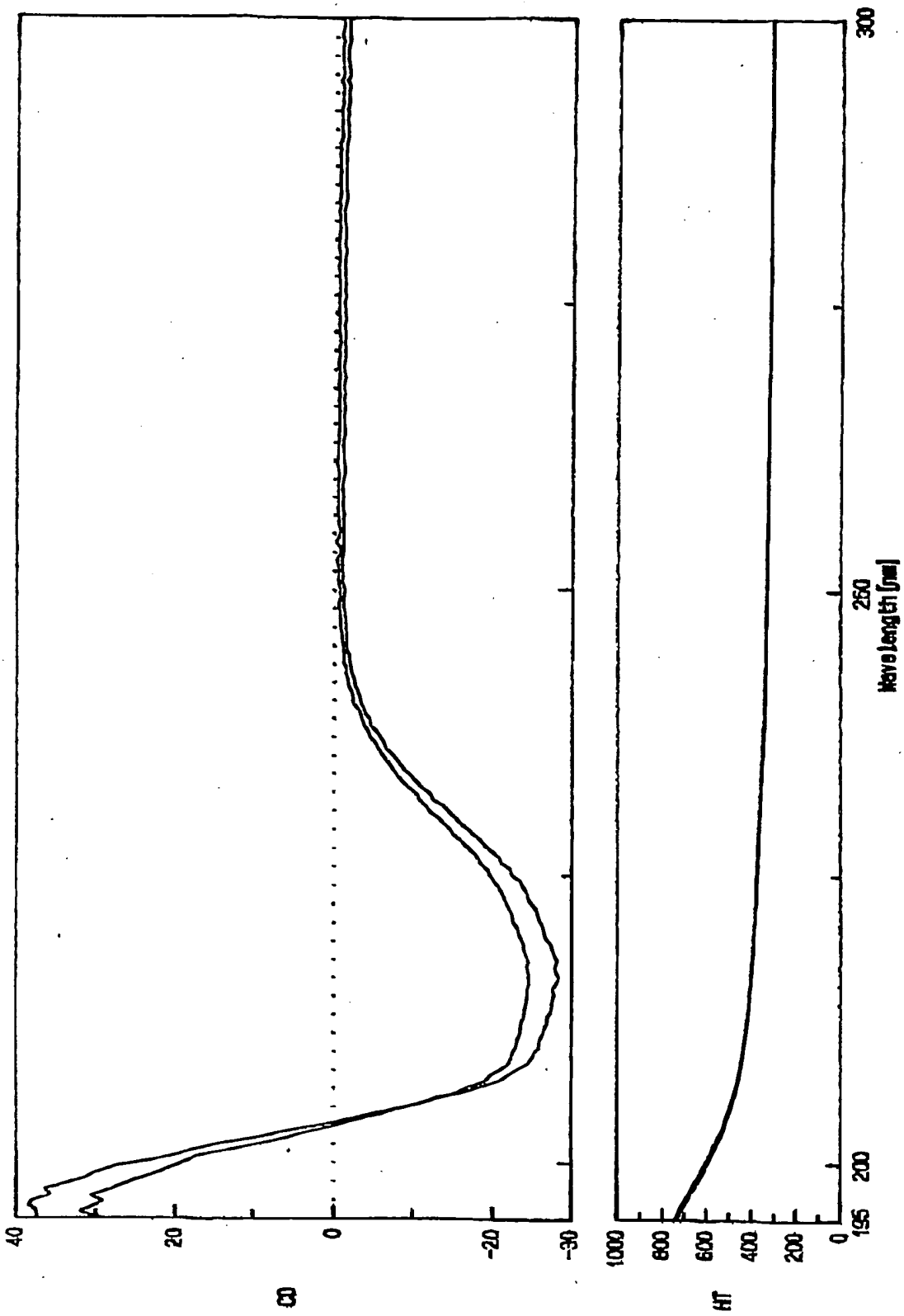

40
20
0
-20
-30
CD
1000
800
600
400
200
0
HT
195
200
250
300
Wavelength [nm]
6798a r Betv 2801
6797a 3xMutant 2595

FIG. 8

Fig. 9

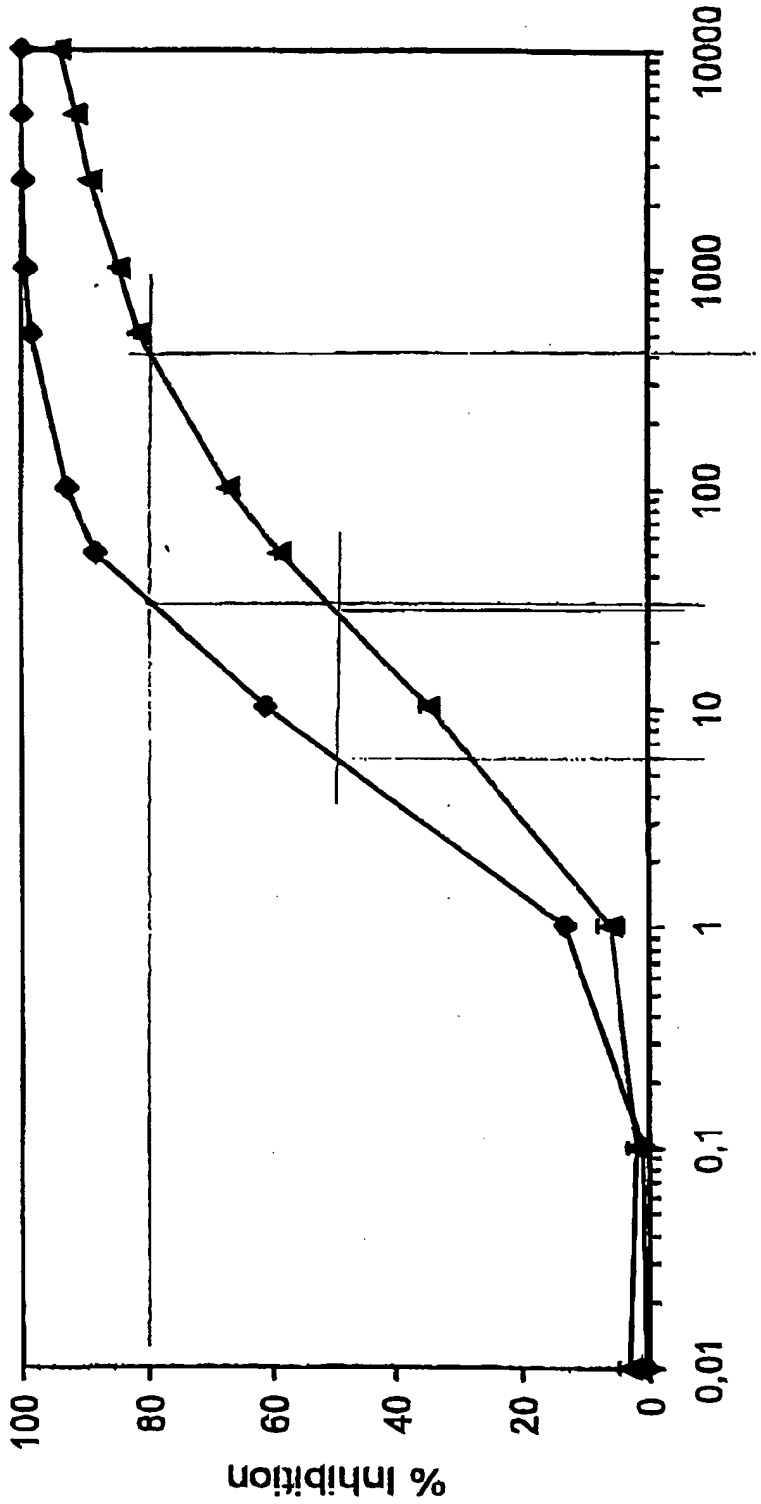
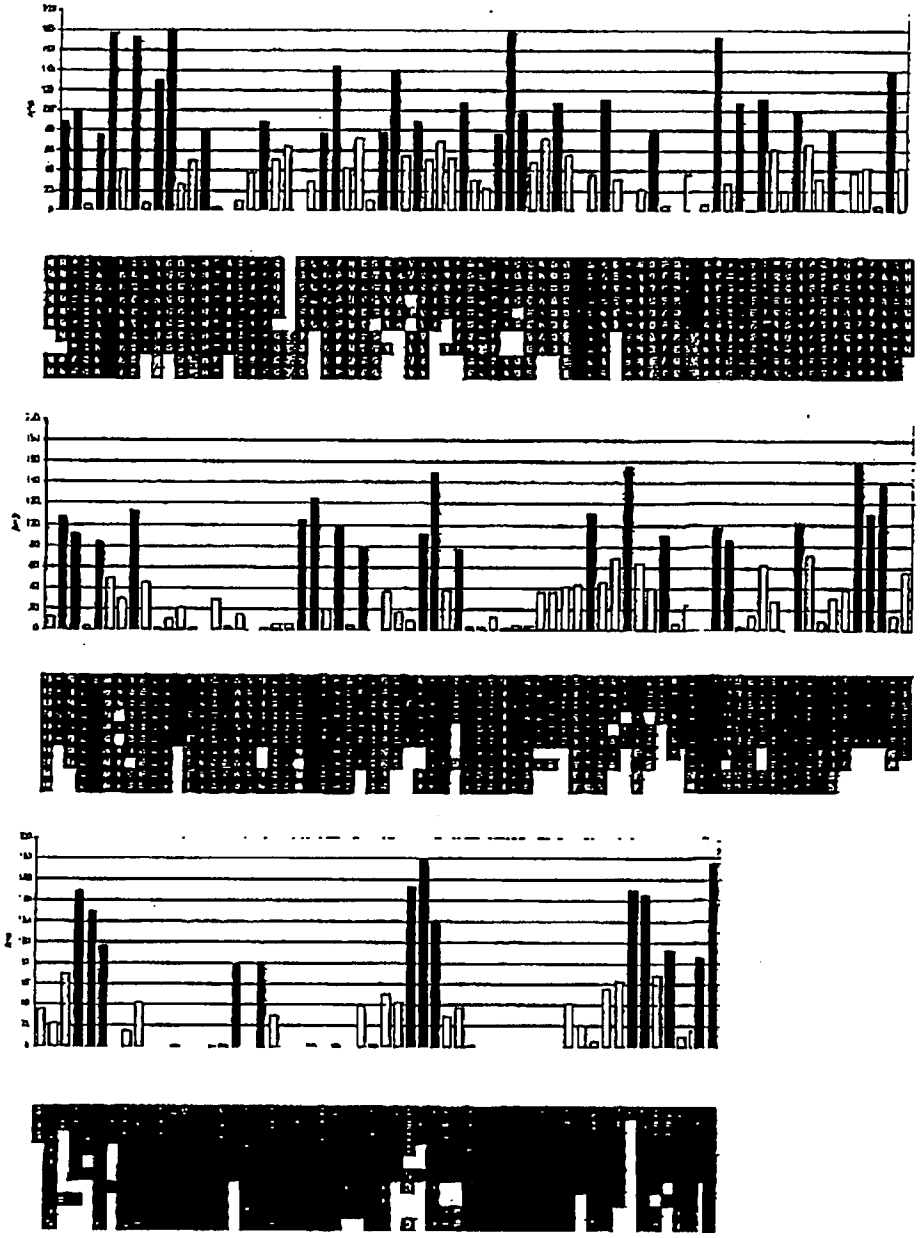

% Inhibition
100
80
60
40
20
0
0,01
0,1
1
10
100
1000
10000
ng inhibitor
Bet v 1
Glu45Ser, Pro108Gly, Asn28Thr+Lys32Gln.

FIG. 9



Conserved residues among Vespula antigen 5

FIG. 10

Fig.11

Mutant-specific oligonucleotide primers used for Ves v 5 mutants. Mutated nucleotides underlined.

Ves v 5 mutant 1 (K72A)

Ves v 5 sense	5´-	ACCACAGCCTCCAGCGAAGAATATGAAAAATTTGGTATGGA -3´
Ves v 5 non-sense	3´-	TGGTGTCGGAGGTCGCTTCTTATACTTTTTAAACCATACCT -5´
sense primer	5´-	CCAGCGGCTAATATGAAAAAT -3´
non-sense primer	3´-	GTCGGAGGTCGCCGATTATAC -5´

Ves v 5 mutant 2 (Y96A)

Ves v 5 sense	5´-	GGCTAATCAATGTCAATATGGTCACGATACTTGCAGGGATG -3´
Ves v 5 non-sense	3´-	CCGATTAGTTACAGTTATACCAGTGCTATGAACGTCCCTAC -5´
sense primer	5´-	TGTCAAGCTGGTCACGATACT -3´
non-sense primer	3´-	TTAGTTACAGTTCGACCAGTG -5´

Fig. 12

Oligonucleotide primers for site directed mutagenesis of Ves v 5.

all sense 1: XhoI start, 38-mer:

EcoRI
5´-CCGCTCGAGAAAAGAAACAATTATTGTAAAATAAAATG
L E K R N N Y C K I K .
Kex2 cleavage site amino terminus of Ves v 5

1	sense	1: K72As	21-mer	5´-CCAGCGGCTAATATGAAAAAT
1	non-sense	2: K72Aa	21-mer	5´-CATATTAGCCGCTGGAGGCTG
2	sense	3: Y96As	21-mer	5´-TGTCAAGCTGGTCACGATACT
2	non-sense	4: Y96Aa	21-mer	5´-GTGACCAGCTTGACATTGATT
all	non-sense	7: CT-pPICZαA,	21-mer	5´-ATTCATCAGCTGCGAGATAGG

Fig. 13

Overview of Ves v 5 mutations

```
   1 AACAATTATTGTAAAATAAAATGTTTGAAAGGAGGTGTCCATACTGCCTGCAAATATGGA   60
   1  N  N  Y  C  K  I  K  C  L  K  G  G  V  H  T  A  C  K  Y  G    20

  61 AGTCTTAAACCGAATTGCGGTAATAAGGTAGTGGTATCCTATGGTCTAACGAAACAAGAG  120
  21  S  L  K  P  N  C  G  N  K  V  V  V  S  Y  G  L  T  K  Q  E    40

 121 AAACAAGACATCTTAAAGGAGCACAATGACTTTAGACAAAAAATTGCACGAGGATTGGAG  180
  41  K  Q  D  I  L  K  E  H  N  D  F  R  Q  K  I  A  R  G  L  E    60

                                    1 [K72A] (AAG-GCT)
 181 ACTAGAGGTAATCCTGGACCACAGCCTCCAGCGAAGAATATGAAAAATTTGGTATGGAAC  240
  61  T  R  G  N  P  G  P  Q  P  P  A  K  N  M  K  N  L  V  W  N    80

                                                2 [Y96A] (TA-GC)
 241 GACGAGTTAGCTTATGTCGCCCAAGTGTGGGCTAATCAATGTCAATATGGTCACGATACT  300
  81  D  E  L  A  Y  V  A  Q  V  W  A  N  Q  C  Q  Y  G  H  D  T   100

 301 TGCAGGGATGTAGCAAAATATCAGGTTGGACAAAACGTAGCCTTAACAGGTAGCACGGCT  360
 101  C  R  D  V  A  K  Y  Q  V  G  Q  N  V  A  L  T  G  S  T  A   120

 361 GCTAAATACGATGATCCAGTTAAACTAGTTAAAATGTGGGAAGATGAAGTGAAAGATTAT  420
 121  A  K  Y  D  D  P  V  K  L  V  K  M  W  E  D  E  V  K  D  Y   140

 421 AATCCTAAGAAAAAGTTTTCGGGAAACGACTTTCTGAAAACCGGCCATTACACTCAAATG  480
 141  N  P  K  K  K  F  S  G  N  D  F  L  K  T  G  H  Y  T  Q  M   160

 481 GTTTGGGCTAACACCAAGGAAGTTGGTTGTGGAAGTATAAAATACATTCAAGAGAAATGG  540
 161  V  W  A  N  T  K  E  V  G  C  G  S  I  K  Y  I  Q  E  K  W   180

 541 CACAAACATTACCTTGTATGTAATTATGGACCCAGCGGAAACTTTAAGAATGAGGAACTT  600
 181  H  K  H  Y  L  V  C  N  Y  G  P  S  G  N  F  K  N  E  E  L   200

 601 TATCAAACAAAGTAA                                               612
 201  Y  Q  T  K  stop                                              204
```

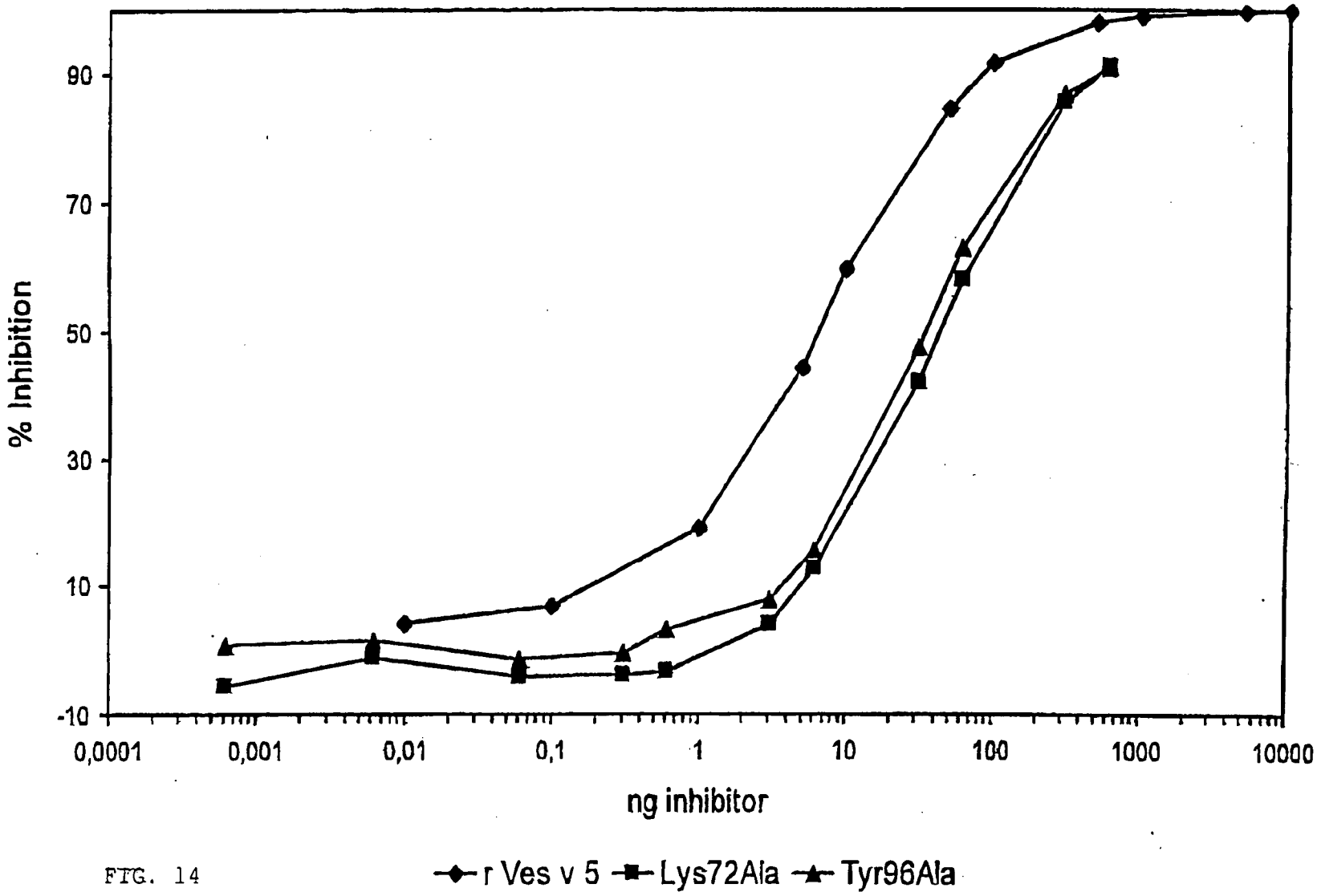

% Inhibition
90
70
50
30
10
-10
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
ng inhibitor
r Ves v 5
Lys72Ala
Tyr96Ala

FIG. 14